PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :		(11) International Publication Number: WO 91/16917
A61K 37/02	A1	(43) International Publication Date: 14 November 1991 (14.11.91)

(21) International Application Number: PCT/US91/02620

(22) International Filing Date: 19 April 1991 (19.04.91)

(30) Priority data:
517,632 2 May 1990 (02.05.90) US

(60) Parent Application or Grant
(63) Related by Continuation
US 517,632 (CIP)
Filed on 2 May 1990 (02.05.90)

(71) Applicant *(for all designated States except US)*: THE UPJOHN COMPANY [US/US]; 301 Henrietta Street, Kalamazoo, MI 49001 (US).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)* : ROBERT, André [US/US]; 929 N. Dartmouth, Kalamazoo, MI 49007 (US).

(74) Agent: DELUCA, Mark; Corporate Patents & Trademarks, The Upjohn Company, Kalamazoo, MI 49001 (US).

(81) Designated States: AT (European patent), AU, BB, BE (European patent), BF (OAPI patent), BG, BJ (OAPI patent), BR, CA, CF (OAPI patent), CG (OAPI patent), CH (European patent), CM (OAPI patent), DE (European patent), DK (European patent), ES (European patent), FI, FR (European patent), GA (OAPI patent), GB (European patent), GR (European patent), HU, IT (European patent), JP, KP, KR, LK, LU (European patent), MC, MG, ML (OAPI patent), MR (OAPI patent), MW, NL (European patent), NO, PL, RO, SD, SE (European patent), SN (OAPI patent), SU, TD (OAPI patent), TG (OAPI patent), US.

Published
With international search report.

(54) Title: METHOD OF TREATING GASTRIC ULCERS AND OBESITY

(57) Abstract

A method for the treatment or prevention of gastric ulcers in mammals is disclosed. This method comprises administering to a mammal, who is suffering from or is particularly susceptible to said gastric ulcers, an amount of Interleukin-1 effective to cure or prevent said gastric ulcers by inducing a cytoprotective effect. Additionally, a method of treating obesity in mammals is disclosed. The method comprises administering to a human suffering obesity, an effective amount of IL-1 to retard gastric emptying.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	**ES**	Spain	**MG**	Madagascar
AU	Australia	**FI**	Finland	**ML**	Mali
BB	Barbados	**FR**	France	**MN**	Mongolia
BE	Belgium	**GA**	Gabon	**MR**	Mauritania
BF	Burkina Faso	**GB**	United Kingdom	**MW**	Malawi
BG	Bulgaria	**GN**	Guinea	**NL**	Netherlands
BJ	Benin	**GR**	Greece	**NO**	Norway
BR	Brazil	**HU**	Hungary	**PL**	Poland
CA	Canada	**IT**	Italy	**RO**	Romania
CF	Central African Republic	**JP**	Japan	**SD**	Sudan
CG	Congo	**KP**	Democratic People's Republic of Korea	**SE**	Sweden
CH	Switzerland			**SN**	Senegal
CI	Côte d'Ivoire	**KR**	Republic of Korea	**SU**	Soviet Union
CM	Cameroon	**LI**	Liechtenstein	**TD**	Chad
CS	Czechoslovakia	**LK**	Sri Lanka	**TG**	Togo
DE	Germany	**LU**	Luxembourg	**US**	United States of America
DK	Denmark	**MC**	Monaco		

METHOD OF TREATING GASTRIC ULCERS AND OBESITY

FIELD OF THE INVENTION

The present invention relates to the prevention and treatment of gastric ulcers and obesity.

BACKGROUND OF THE INVENTION

The total number of patients in the United States who have gastric disease is not known. However, over 100,000 patients with chronic benign gastric ulcers are hospitalized each year. Studies from Denmark suggest that the incidence of new gastric ulcers occurring yearly in all persons over 15 years of age is 5 per 10,000 persons. Gastric ulcers rarely develop before the age of 40 years and the peak incidence occurs from ages 55 to 65 years. The incidence is approximately the same for men as for women.

Overall, hospitalization for and deaths from peptic ulcer disease have decreased during the past 10 to 20 years. However, the decrease in hospitalizations is due almost exclusively to a reduction in hospital admissions for duodenal ulcer rather than for gastric ulcer. In fact, overall hospitalizations for gastric ulcer have remained stable during the past several years even though admissions for uncomplicated ulcers have decreased slightly. Outpatient visits for duodenal ulcer also have declined, whereas by comparison outpatient visits for gastric ulcer have decreased minimally.

The reasons for the reduced incidence of duodenal ulcers are not known nor is it clear why hospitalizations or outpatient visits for gastric ulcers have not decreased to the same degree. Perhaps it is either because the pathogenesis of the two diseases is different, because new treatments for ulcer disease are more specific for duodenal than for gastric ulcer, or because gastric ulcers occur in older patients, who are more prone to concomitant diseases requiring hospitalizations.

Benign gastric ulcers can occur anywhere in the stomach, although the most frequent location is on the lesser curvature near the angularis. Chronic ulcers are usually round. However, they may be oval, elongated, or elliptical. They have depth and penetrate the muscularis mucosae, a feature that distinguishes them from acute superficial erosions, as might occur in patients with stress from severe medical or surgical illnesses. Gastric ulcers vary in greatest dimension from a few millimeters to several centimeters but most are between 1 and 2 cm when diagnosed by endoscopy or X-ray. A single ulcer is the rule, although two or more may be found in some patients.

Duodenal ulcer is a break in the mucosa of the duodenum extending through the muscularis mucosae. The histopathology of duodenal ulcer is similar to that of gastric ulcer. Duodenal ulcer is a common disease. In 1975 it was estimated that approximately 4 million people in the United States suffered from ulcer (both gastric and duodenal), with a direct cost (i.e., hospital care, physician costs, drug costs) of about 1.2 billion dollars and indirect costs (i.e., time loss from work

plus loss of lifetime earnings in the event of ulcer death) of 1.5 billion dollars. Therefore, peptic ulcer (both gastric and duodenal) accounted for about 10 percent of the total medical costs for digestive diseases, which were estimated for 1975 to be 25 billion dollars.

Obesity is a major problem in the United States. In many cases the health of obese persons is sufficiently compromised to become life threatening.

Interleukin-1 (IL-1), a 17,500 dalton protein, is one of several cytokines isolated from certain cells, particularly lymphocytes, thymocytes, macrophages, endothelial cells, synovial cells, and keratinocytes. It has been sequenced, cloned and expressed in E. coli. IL-1 is one of the mediators of inflammation and may be responsible for the fever that accompanies infections. It induces accumulation of neutrophiles in inflamed areas and peripheral blood; promotes leukocytic adhesion to endothelium; is mitogenic for thymocytes; stimulates the pituitary to secrete ACTH in vitro and in vivo, resulting in an increase in serum corticosterone level; and, prevents development of colitis in rabbits. It also stimulates synthesis of prostaglandin E_2 (PGE_2) in isolated dermal and synovial cells, an effect due to increased synthesis of the enzyme cyclooxygenase, and promotes formation of PGE_2 by the rabbit colon.

The present invention provides a method of treating gastric ulcers comprising administration of an effective amount of IL-1. The present invention additionally provides a method of preventing gastric ulcers comprising administration of an effective amount of IL-1. IL-1 stimulates the synthesis of prostaglandin E_2 (PGE_2). It is believed by stimulating production of PGE_2 in the gastric mucosa, the administration of IL-1 may protect the gastric mucosa against damage associated with gastric ulcers.

The present invention provides a method of treating obesity comprising administration of an effective amount of IL-1. The present invention additionally provides a method of preventing obesity comprising administration of an effective amount of IL-1. IL-1 retards emptying of gastric contents. By retarding emptying of gastric contents, the administration of IL-1 reduces a patient's desire for food and this is helpful in weight loss programs.

INFORMATION DISCLOSURE

Uehara, A., et al., Biochem. and Biophys. Res. Comm., Vol. 162, 3:1578-1584 (August 15, 1989) disclose that Interleukin-1, both endogenously released and exogenously administered, suppresses gastric acid secretion and that the Interleukin-1-induced inhibition of acid output is possibly mediated by prostaglandin.

Dinarello, C. A., FASEB J. 2:108-115, 1988, provides a review article on the biology of Interleukin-1. Dinarello reports that IL-1 triggers the synthesis of PGE_2, in particular, in cultured endothelial cells. Additionally, Dinarello reports that in vitro, IL-1 induces synovial cells and chondrocyte to produce PGE_2.

McCarthy, D. O., et al., Am. J. Clin. Nutr. 42:1179-1182, December 1985, disclose that

the loss of food appetite commonly associated with infectious illnesses where high fever is a symptom is due to elevated levels of Interleukin-1. McCarthy et al. report that infection-induced anorexia is, in part, due to the release of Interleukin-1.

Cominelli, F., et al., J. Clin. Invest. 85:582-586, February 1990, report that treatment with low-dose IL-1 has protective effects in animal models of inflammation and tissue injury. It is disclosed that Interleukin-1 suppresses inflammation in rabbit colitis.

Robertson, B., et al., J. Invest. Dermatol. 88:380-387, 1987, disclose that prostaglandins are involved in the depression of the capacity of normal mice to elicit contact hypersensitivity responses when administered Interleukin-1 in vivo.

Dinarello, C. A. et al., J. Clin. Invest. 77:1734-1739, June 1986, discuss multiple biological activities of human recombinant Interleukin-1. It is reported that human recombinant Interleukin-1 was shown to be a potent inflammatory agent by its ability to induce human dermal fibroblast prostaglandin E_2 production in vitro and to produce monophasic (endogenous pyrogen) fever when injected into rabbits or endotoxin-resistant mice.

U. S. Patent No. 4,816,436, issued March 28, 1989, to Jacobs discloses compositions for treating arthritis or inflammation in mammals, including humans, containing Interleukin-1. The compositions are administered by intra-articular, i.m., i.v., or i.p. injection at doses of 10,000 to 1,000,000,000 U/kg.

South African Patent No. 8501996, issued September 20, 1985, to Cerretti, D. P., et al., discloses homogeneous Interleukin-1. Recombinant DNA procedures useful for making large quantities of IL-1 are disclosed. IL-1 may be used to treat autoimmune diseases such as arthritis and lupus erythematosis and for wound and burn healing.

PCT Publication No. WO8905653-A, published June 20, 1989, refers to a topical composition containing mammalian Interleukin-1 in a hydrophilic vehicle useful for promoting wound healing in mammals. The topical composition can be used to treat patients with chronic bedsores, ulcerative skin conditions, diabetes or other metabolic diseases. Additionally, the composition can be used to treat those who are aged, malnourished, immunodeficient, undergoing corticosteriod or chemotherapy treatment and those who abuse chemical substances.

European Patent Application No. EP-188864-A, published July 30, 1986, discloses pure DNA extracted from human cells coding for human Interleukin-1. The entire DNA coding sequence and amino acid sequences are disclosed. Recombinant DNA cloning vectors containing the IL-1 gene and hosts transformed with these cloning vectors are also disclosed. The cultivated transformants produce Interleukin-1 which is useful for treating arthritis, lupus erythematosus, wounds and burns.

SUMMARY OF THE INVENTION

The present invention provides a method for the treatment or prevention of gastric ulcers

in mammals. This method comprises administering to a mammal, who is suffering from or is particularly susceptible to said gastric ulcers, an amount of Interleukin-1 effective to cure or prevent said gastric ulcers. Additionally, the present invention provides a method of treating obesity in mammals. The method comprises administering to a human suffering obesity, an effective amount of IL-1 to retard gastric emptying.

DETAILED DESCRIPTION OF THE INVENTION

Human recombinant Interleukin-1 (IL-1) was administered intraperitoneally to rats. The following effects were observed:

1. IL-1ß is cytoprotective for the stomach. It prevented gastric necrosis produced by oral administration of 1 ml of absolute ethanol to fasted animals. The ED_{50} (dose reducing the number of lesions by 50%) was 12000 units/kg corresponding to 110 ng/animal. IL-1ß is 125 times more potent than prostaglandin E_2 as a cytoprotective agent.
2. IL-1α is also cytoprotective, half as active as IL-1ß.
3. The cytoprotective effect of IL-1 is blocked by both indomethacin (inhibitor of PG synthesis) and by IRAP (selective IL-1 receptor antagonist).
4. IL-1 stimulates prostaglandin synthesis by the stomach. A dose of 30,000 units/kg increased the formation of PGE_2 by the gastric mucosa by 35%.
5. IL-1 prevents formation of gastric erosions induced by aspirin. The ED_{50} was 10,000 units/kg.
6. IL-1 retards gastric emptying. The ED_{50} (dose delaying gastric emptying by 50%) was 2000 units/kg.

IL-1 is the most potent of known agents as gastric cytoprotective and antiulcer. It appears to act by stimulating the synthesis of prostaglandins by the stomach. IL-1 also plays a role in gastric motility.

These studies suggest that the stomach possesses IL-1 receptors. These are probably located on parietal cells (that produce acid), prostaglandin-producing cells, smooth muscle cells (that contract to empty the gastric contents), and yet unidentified gastric cells involved in cytoprotection. Interleukin-1 receptor antagonist protein (IRAP) inhibits the cytoprotective action of IL-1, but does not inhibit cytoprotection induced by PGE_2.

These findings show that IL-1 is extremely potent in protecting the gastric mucosa against damage produced by a necrotizing agent (absolute ethanol), in preventing aspirin-induced gastric erosions, and in retarding gastric emptying. We know of no other substance, natural of synthetic, that even approaches the potency of IL-1.

Accordingly, gastric ulcers may be prevented or treated in mammals by administering an amount of Interleukin-1 sufficient to demonstrate a cytoprotective effect. In addition, obesity may

be treated in mammals by administrating an amount of Interleukin-1 sufficient to reduce gastric motility. By reducing gastric mobility, food will remain in the stomach longer and thereby, reduce the appetite of the patient.

The efficacy of IL-1 as a cytoprotective agent useful in the prevention and treatment of gastric ulcers as well as its usefulness in the treatment of obesity is demonstrated by the data presented.

Female Sprague-Dawley rats of an average body weight of 210 g were used. In all experiments they were fasted overnight in individual cages to prevent coprophagy.

Human recombinant Interleukin-1ß (hrIL-1) is a 17,500 dalton protein. IL-1 has been assayed by a method based on the ability of IL-1 to generate IL-2 from a phytohemagglutinin-activated murine T cell line, LBRM-33-1A5. IL-2 was assayed by measuring the incorporation of methyl-^{3}H-thymidine into HT-2 cells as a measure of cell proliferation. The potency of the IL-1 was 3.6×10^{7} LAF (lymphocyte activating factor) units per mg protein. It was supplied in a buffer containing 2.26 mg/ml. In this report, "IL-1" refers to IL-1ß.

Recombinant human Interleukin-1 alpha (hrIL-1α), an 18 Kd protein, was used. Its potency was 2×10^{7} LAF units per mg protein. It was supplied in a buffer containing 1 mg/ml.

IRAP (human recombinant Interleukin-1 receptor antagonist protein) is a 17,143 dalton protein derived from U937 cells. It has been sequenced, cloned and expressed in E. coli. IRAP is an active competitive inhibitor of the binding of IL-1 to a T cell form of the IL-1 receptor. One IRAP unit is defined as the amount required to inhibit one unit of IL-1 by 50% in the 1A5/HT-2 cell line assay, using the standard murine LAF (lymphocyte activator factor) unit for IL-1. The IRAP used was assayed at 2.3×10^{4} units per mg protein; it was supplied in a buffer solution containing 30 mg/ml.

IL-1α, IL-1ß, and IRAP were administered intraperitoneally (IP) in 0.5 ml of 0.5% rat serum diluted in saline. Rat serum was obtained from the aortic blood of a fed rat.

IL-1α and IL-1ß were studied to determine the ability as potential cytoprotective agents.

IL-1ß was injected IP at doses ranging from 2000 to 60,000 units/kg (corresponding to 100 to 3000 ng/kg). One hour later, 1 ml of 100% (absolute) ethanol was given orally. That dose of ethanol, when given alone, produces extensive and deep necrosis of large segments of the gastric mucosa. The animals were killed with CO_2 one hour after ethanol, the stomachs were dissected out, opened along the greater curvature, and randomized. Using a 2 X binocular magnifier, the necrotic lesions were counted without knowledge of the treatment given, and the average number of lesions per stomach was calculated for each group. The lesions appeared as wide and elongated red streaks in the corpus mucosa. Histologically, they consist of hemorrhages and extensive necrosis of the mucosa and edema of the submucosa.

In order to determine how specific the cytoprotective effect of IL-1ß was, IL-1α was tested. IL-1α was injected IP to fasted rats at doses ranging from 1000 to 100,000 units/kg (50 to 5000 ng/kg), one hour before oral administration of 1 ml of 100% ethanol. The animals were killed one hour after 100% ethanol, and the average number of gastric necrotic lesions per stomach was recorded in a blind fashion.

Both IL-1α and IL-1ß were cytoprotective. They decreased the number of ethanol-induced gastric mucosal lesions dose dependently, with an ED_{50} of 25,000 units/kg (1250 ng/kg) for IL-1α and 12,000 units/kg (600 ng/kg) for IL-1ß. By comparison, the parenteral cytoprotective ED_{50} of PGE_2 is 75 μg/kg, making IL-1ß 125 times more potent than PGE_2. At a dose of IL-1 of 30,000 units/kg (1500 ng/kg), nearly no lesions were visible.

To find out whether IL-1ß might be cytoprotective by direct contact with the mucosa, it was administered orally at a dose of 108,000 units/kg (3000 ng/kg), 30 minutes before oral administration of 1 ml 100% ethanol. The animals were killed one hour after 100% ethanol administration and the gastric lesions were counted. In a separate experiment, omeprazole, an antisecretory compound that elevates the pH of gastric juice to 7 in one hour, was injected subcutaneously at a dose of 10 mg/kg, in saline suspension containing a drop of Tween 80, two hours before IL-1. We used omeprazole to reduce the degradation of IL-1 that is likely to occur when mixed with acid juice which also contains pepsin. By neutralizing the juice, pepsin (optimal pH is 1.5) is inactivated; this should prevent the enzymatic degradation of IL-1. IL-1 was inactive, with or without omeprazole.

The role of endogenous prostaglandins in IL-1-induced cyto-protection was studied.

Indomethacin, 10 mg/kg, was injected subcutaneously in 1 ml of 1% $NaHCO_3$, 90 minutes before IL-1. IL-1 was given IP at a dose of 30,000 units/kg (1500 ng/kg). This corresponds to 5900 units/rat (295 ng/rat). One hour after IL-1, 1 ml of 100% ethanol was given orally, and the animals were killed one hour after ethanol. The gastric lesions were counted. Gastric cytoprotection induced by a dose of 30,000 units/kg (1500 ng/kg) of IL-1 against 100% ethanol was blocked by prior treatment with indomethacin (10 mg/kg) administered subcutaneously 90 minutes before IL-1.

IL-1 was injected IP at a dose of 30,000 units/kg (1500 ng/kg). One group of animals was killed after one hour, and another group after 2 hours. The gastric mucosa was dissected out and processed for radioimmunoassay of PGE_2 and 6-keto $PGF_1\alpha$ (a metabolite of prostacyclin). The results were expressed as ng per gram of wet tissue. IL-1, 30,000 units/kg (1500 ng/kg) injected IP one or two hours before killing, stimulated PGE_2 generation by the gastric mucosa by 38% and 33%, respectively. IL-1 did not affect the formation of 6-keto $PGF_1\alpha$.

IRAP was administered IP at doses of 11,500, 23,000 and 46,000 units/kg (50, 100 and 200 μg/kg, respectively), 10 minutes before a IP injection of 30,000 units/kg of IL-1 (1500 ng/kg).

One hour after IL-1, 1 ml of 100% ethanol was given orally, and the animals were killed one hour later. The gastric lesions were counted. IRAP, given IP ten minutes prior to a dose of 30,000 units/kg (150 μg/kg) of IL-1, inhibited the cytoprotective effect of IL-1 dose dependently, with an ED_{50} of about 23,000 units/kg (100 μg/kg), or 437 units/animal (19 μg/animal). A dose of 46,000 units/kg (200 μg/kg) completely blocked the cytoprotective effect of IL-1. IRAP, 115,000 units/kg (500 μg/kg), did not prevent cytoprotection produced by PGE_2 given either orally (100 μg/kg) or subcutaneously (150 μg/kg).

The specificity of IL-1 as a cytoprotective agent was demonstrated by the finding that an IL-1 receptor antagonist (IRAP) blocked the protective effect of IL-1. Since, however, the protection by IL-1 was also prevented by administration of indomethacin, an inhibitor of prostaglandin synthesis, cytoprotection by IL-1 appears to be mediated by the synthesis and release of prostaglandins by the stomach. This conclusion is supported by the 35% increase in PGE_2 generation by the stomach following administration of IL-1 at a cytoprotective dose.

The finding that gastric cytoprotection by PGE_2 was not prevented by IRAP also suggests that IRAP is specific for IL-1, and does not bind to receptors involved in gastric cytoprotection. This selective effect of IRAP (blocking cytoprotection produced by IL-1 but not that produced by PGE_2) further supports the interpretation that PGE_2, and possibly other prostaglandins, act as second messengers which may mediate IL-1-induced cytoprotection.

It is significant that both IL-1α and IL-1ß were cytoprotective, IL-1ß being twice as potent as IL-1α. This is consistent with some other properties shared by these two cytokines, such as mitogenicity for mononuclear and spleen cells in vitro, increase in serum corticosterone and development of neutrophilia in mice.

Since IL-1 and IRAP are natural substances, on can surmise that they may both play a role in modulating gastric functions and in maintaining gastric mucosal integrity. In this regard, it would be important to determine whether IL-1 and IRAP are present in the gastric mucosa, and if so, in which cells. However, even if they were not made by gastric cells but originated in other cells of the body, their biological half lives in the blood is long enough to explain an effect on the stomach. Another in vivo effect of IRAP is the inhibition of the rise of serum corticosterone after administration of IL-1.

Since IL-1 is cytoprotective, one would have expected that administration of an antagonist to IL-1 would be deleterious. However, gastric lesions did not develop after injection of IRAP alone, not did IRAP aggravate the necrotic lesions produced by absolute ethanol. It is more likely that these substances (IL-1 and IRAP) act in conjunction with other mediators in modulating the natural defense of the gastric mucosa.

Although addition of IL-1 to dermal and synovial cells in culture is known to stimulate the release of PGE_2 in the incubation medium, to our knowledge, in vivo stimulation of PGE_2

synthesis after administration of IL-1 to animals has not been reported.

To determine the effect of IL-1 on aspirin-induced gastric erosions, aspirin, 50 mg/kg suspended in 1 ml water with a drop of Tween 80, was given orally. The animals were killed one hour later, and the gastric erosions were scored (average number per stomach) without knowledge of the treatment given. IL-1 was administered IP at doses of 10,000 and 30,000 units/kg (500 and 1500 ng/kg, respectively), one hour before aspirin. IL-1 inhibited, dose dependently, gastric erosions produced by aspirin. The ED_{50} (dose reducing the average number of erosions by 50%) was around 10,000 units/kg (500 ng/kg), corresponding to 1900 units/animal (95 ng/animal).

The antiulcer effect of IL-1 (on aspirin-induced erosions) may have a dual mechanism: reduction of acid secretion and cyto-protection. Aspirin requires the presence of acid in the stomach to be ulcerogenic, which explains why antisecretory agents prevent aspirin-induced lesions. Since IL-1 also inhibits acid secretion, it is likely to demonstrate an antiulcer effect as a consequence of the antisecretory effect. However, IL-1 is also cytoprotective, i.e., it protects the gastric mucosa by a mechanism independent of inhibition of gastric secretion; therefore, the antiulcer effect could also be due to such property.

The effect of IL-1 on gastric emptying was studied. This study was performed in animals fed ad libitum. Since rats are nocturnal eaters, their stomachs are filled with food in the morning. At 8:00 AM, a group of animals was killed, and their stomachs were dissected out, weighed and opened. The contents were removed, the stomachs were rinsed and weighed again. The difference between the two weights corresponds to the amount of food present in the stomach. In other groups, food and water were removed at 8:00 AM, and IL-1 was injected IP at doses ranging from 1000 to 30,000 units/kg (50-1500 ng/kg), corresponding to 222-6660 units/rat (11.1 to 333 ng/rat). Controls received the vehicle. The animals were killed four hours after IL-1 (12:00 PM), and the stomachs were weighed, full and after removing the contents.

IL-1 retarded gastric emptying dose dependently. The ED_{50} (dose retarding gastric emptying by 50%) was 10,000 units (500 ng/kg), corresponding to 2220 units/animal (111 ng/animal). At 30,000 units/kg (1500 ng/kg), gastric emptying was totally prevented.

Gastric emptying of solids is due to the combined effect of contractions of gastric smooth muscle (mostly of the antrum) and relaxation of the pyloric sphincter.

IL-1 was reported to reduce spontaneous food intake in rats at a dose of 640,000 unit/animal (300,000 units/kg) and it was suggested that the anorexia associated with infections may be due to the release of IL-1. In view of the retardation of gastric emptying by IL-1 reported here, it is equally plausible that the anorexia of fever is related to delayed gastric emptying caused by IL-1. Appetite is likely to be lost when the stomach remains filled.

The data reported here suggest that the stomach possesses IL-1 receptors on several cells:

a) parietal cells, as suggested by the antisecretory effect.

b) cells responsible for cytoprotection (these cells have not been identified),

c) cells that synthesize prostaglandins (parietal cells, mast cells), as suggested by the stimulation of prostaglandin synthesis by the gastric mucosa, and

d) smooth muscle cells, as suggested by the retardation in gastric emptying.

Other natural substances have been proposed as playing a role in gastric pathology. In particular, platelet activating factor and endothelin were reported to sensitize the gastric mucosa to damage. We are not aware, however, of natural substances other than the prostaglandins that exert a gastric protective effect against necrotizing agents. In this regard, IL-1 is more potent than natural prostaglandins. If IL-1 acts primarily through endogenous release of prostaglandins, the finding that it is much more active than PGE_2 may be due to a sustained release of prostaglandins, as opposed to the effect of bolus administration of these substances that produces short-live peak levels in the gastric tissue.

Example 1 Use of IL-1 in the Treatment of Gastric Ulcers

IL-1 is used in humans with affections of the esophagus, the stomach and the duodenum, characterized by inflammation and ulcerations. Such affections include esophagitis, gastritis, duodenitis, peptic ulcer, Zollinger-Ellison syndrome, reflux esophagitis, and other ailments related to mal-digestion and hyperacidity.

Such patients complain of pain, abdominal discomfort, bloating, nausea and eructation. The diagnosis is established by clinical symptoms and by signs. The latter include visualization of the mucosal defect (ulcer, inflammation of the mucosa, esophagitis, gastritis, duodenitis) with the aid of X-rays and endoscopy. Bleeding, either through vomiting or the passage of occult blood, is also another sign of mucosal defect.

IL-1 is formulated either as a solution in saline or buffer at physiological pH, or as an emulsion similar to that used for administration of antibiotics. The purpose of such emulsions is to retard the absorption and the degradation of IL-1. It can also be mixed with adjuvants for the same purpose. It could also be formulated as an aerosol for intra-nasal administration.

IL-1 is administered at doses ranging from 5 micrograms to 1 milligram per treatment, and the frequency of treatments varies from once a day to four times a day. When given several times a day, it is preferentially administered before meals in order to reduce secretion of gastric acid occurring in response to food.

The routes of administration include subscutaneous and intramuscular injection, oral administration, preferentially as enteric coated preparations, and intra-nasal aerosol.

Example 2 Use of IL-1 for the Treatment of Obesity

For the treatment of obesity, IL-1 is given in similar formulations and following the same schedule of treatment as described in Example 1. Patients treated are obese individuals, as well as patients who are overweight without being overly obese. IL-1 is presumed to act by reducing

appetite either by acting on appetite centers in the central nervous system, or by retarding the emptying of food from the stomach. Patients whose stomachs retain food in their stomach for prolonged periods of time do not have a desire to eat; such reduced food intake would contribute in causing weight loss. IL-1 would also be useful in diseases in which increased food intake is a major component, such as diabetes. In such cases, IL-1, by reducing food intake, would be beneficial for the control of diabetes.

CLAIMS

1. A method for the treatment or prevention of gastric ulcers in mammals which comprises:

administering to a mammal, who is suffering from or is particularly susceptible to said gastric ulcers, an amount of Interleukin-1 effective to cure or prevent said gastric ulcers by induction of a cytoprotective effect.

2. A method according to Claim 1 wherein said mammal is human.

3. A method according to Claim 2 wherein said Interleukin-1 is administered prophylactically.

4. A method according to Claim 2 wherein said Interleukin-1 is administered therapeutically.

5. A method according to Claim 2 where said Interleukin-1 is administered subcutaneously.

6. A method according to Claim 5 wherein said amount of Interleukin-1 administered to said human is 15-1500 ng IL-1 per kilogram of human.

7. A method of protecting the gastrointestinal tract in a mammal from the untoward, non-gastric-acid-induced effects to gastrointestinally injurious agents, which comprises:

administering to said mammal an amount of Interleukin-1 effective to induce a cytoprotective effect in the gastrointestinal tract.

8. A method according to Claim 7 wherein said amount of Interleukin-1 administered to said mammal is 15-1500 ng IL-1 per kilogram of mammal.

9. A method according to Claim 8 wherein said mammal is a human.

10. A method according to Claim 1 wherein said Interleukin-1 is administered therapeutically.

11. A method according to Claim 1 where said Interleukin-1 is administered subcutaneously.

12. A method according to Claim 11 wherein said amount of Interleukin-1 administered to said mammal is 15-1500 ng IL-1 per kilogram of mammal.

13. A method for the treatment or prevention of obesity in mammals which comprises:

administering to a mammal, who is suffering from or is particularly susceptible to obesity

an amount of Interleukin-1 effective to retard gastric emptying.

14. A method according to Claim 13 wherein said mammal is human.

15. A method according to Claim 14 wherein said Interleukin-1 is administered prophylactically.

16. A method according to Claim 14 wherein said Interleukin-1 is administered therapeutically.

17. A method according to Claim 14 where said Interleukin-1 is administered subcutaneously.

18. A method according to Claim 17 wherein said amount of Interleukin-1 administered to said human is 15-1500 ng IL-1 per kilogram of human.

19. A method according to Claim 13 where said Interleukin-1 is administered subcutaneously.

20. A method according to Claim 19 wherein said amount of Interleukin-1 administered to said mammal is 15-1500 ng IL-1 per kilogram of mammal.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 91/02620

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[5]: A 61 K 37/02

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[5]	C 07 K, A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
L	World Patent Index (Latest), Accession no. 90-242907, week 32, Derwent Publications Ltd, (London, GB), & JP, A, 2169522 (DAINIPPON PHARM. K.K.) --	
L	Biochemical and Biophysical Research Communications, vol. 162, no. 3, 15 August 1989, Academic Press, Inc., A. Uehara et al.: "Interleukin-1 inhibits the secretion of gastric acid in rats: possible involvement of prostaglandin", pages 1578-1584 see page 1583, paragraph 1 --	
L	Neurosciene Letters, vol. 119, no. 1, 1990, Elsevier Scientific Publishers Ltd, (IE), T. Ishikawa et al.: "The central inhibitory effect of interleukin-1 on gastric acid secretion", pages 114-117 see page 114, paragraph 3	

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
4th July 1991	27. 08. 91
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	M. PEIS M. Peis

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

The documents cited under category L have been included in the search report considering a possible amendment of the claims foreseen in rule 33.3b PCT. From the amendment of the claims a lack of unity of invention could arise.

V. [X] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. [X] Claim numbers 1-20 because they relate to subject matter not required to be searched by this Authority, namely:

Pls. see Rule 39.1 (iv) - PCT:

Method for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.

2. [ ] Claim numbers ....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claim numbers ....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

—

3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees.